



Validated Multi-Residue Method for Extraction and Analysis of Trace-Level Pesticides in Surface Water

Application

Environmental

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Abstract

A USGS analytical method using gas chromatography/mass selective detector (GC/MSD) was modified and used to identify the source of pesticides and to calculate the pesticide load in river water during storm seasons. The method was validated to cover 12 organophosphate pesticides and 5 pyrethroids. The limits of quantitation for these target compounds are 10–500 ppt using a GC/MSD selected ion monitoring (SIM) method.

Introduction

The California Regional Water Quality Control Board has identified the lower San Joaquin River as high priority for development of Total Maximum Daily Loads (TMDL). The California Regional Water Quality Control Board conducted a study to determine a possible source of toxicity in the San Joaquin and Sacramento rivers, especially during

storm events, following the application of dormant sprays (in winter time). Studies are necessary to determine the pesticide level in these waterbodies prior to, during, and after storm events. Analysis will also be required for samples collected prior to the start of dormant season application to determine baseline levels. The data will be used to identify the pesticide source in these waterbodies and to calculate the pesticide load in the river during storm season.

The reference analytical method used for the TMDL program was modified from USGS GC/MSD method (Open File Report 95-181 and 01-273). The method was validated to cover 12 organophosphate pesticides and 5 pyrethroids. The Water Quality Control Board specified the limits of quantification (LOQ).

Instrumentation

Equipment Required

- Balance (up to 2 Kg \pm 1 g)
- 2-L separatory funnel
- 250-mL round bottom flask (boiling flask)
- Glass filter funnel
- Rotavapor evaporator
- 15-mL collection tube
- Nitrogen evaporator
- Vortex
- Sonicator (optional)



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Reagents and Supplies

- Methylene chloride: pesticide grade
- Anhydrous sodium sulfate; Certified A.C.S. 10–60 mesh
- Glass wool: Pyrex brand fiber glass
- NaCl: Certified A.C.S.
- Nylon filter (0.45 µm) (Alltech 2024 or equivalent)
- Surrogate spiking solution: 0.5-ng/mL chlorpyrifos methyl in acetone
- Internal standard (ISTD) solution: anthracene-d₁₀, pyrene-d₁₀, and chrysene-d₁₂ at 0.5 ng/mL (500 ppt): Ultra Scientific ISM-520

6890 GC Parameters

- Column: 30 m × 0.25 mm × 0.25 µm HP-5MS
- Inlet temperature: 230 °C
- Injection volume: 2 µL (splitless)
- Oven ramp:
70(2)/25/150(0)/3/200(0)/8/280(12)

5973 MSD Parameters

- SIM mode
- Max. sensitivity Auto Tune
- SIM groups (dwell time for each ion = 50 ms)

Group	Start, min	# Ions	Ions
1	4.0	7	86, 128, 164, 173, 186, 189, 201
2	15.5	13	88, 115, 125, 137, 144, 179, 186, 188, 201, 274, 286, 288, 304
3	19.5	10	162, 197, 198, 204, 238, 240, 258, 301, 314, 332
4	23.0	3	85, 125, 145
5	26.0	12	77, 132, 135, 141, 160, 165, 166, 173, 181, 209, 240, 350
6	32.0	7	163, 165, 167, 181, 225, 226, 419

Sample Preparation

A 1-L water sample is delivered to the laboratory in an amber-glass bottle. Samples are stored under normal refrigerated condition (approximately 4 °C) until extraction within 7 days.

1. Weigh and record the 1-L water sample, including sediments.
2. Pour the water sample, including sediments, into a 2-L size separatory funnel. Do not filter since pesticides would stick to humic materials.
3. Spike with 1-mL surrogate spiking solution: 0.5-ng/mL (ppb) chlorpyrifos methyl (0.5 ng). Shake the separatory funnel gently to mix.
4. Add 10–15 g granular NaCl for salting-out purpose. Shake gently to dissolve salt.
5. Rinse water sample container with 60-mL methylene chloride and add to the separatory funnel. Weigh and record the empty water sample container. Subtract and record the water sample weight.
6. Shake and release pressure several times. Shake well for 3 minutes. Let settle until the lower methylene chloride layer is completely separated from the above water layer. If there is too much emulsion in the funnel, use a sonicator to break up the emulsion.
7. Filter bottom organic layer through a bed of granular anhydrous sodium sulfate (approx. 20 g) into a 250-mL round bottom flask. The sodium sulfate is supported on glass wool, prewashed with 30–40-mL methylene chloride.
8. Add 60-mL methylene chloride into the funnel and repeat steps 6–7 two more times.
9. The round bottom flask should now have about 180 mL of methylene chloride. Place the round bottom flask on Rotavapor evaporator (at about 100 rpm) and evaporate down to 5–7 mL at 40 °C.
10. Transfer contents of the round bottom flask to a 15-mL collection tube. Rinse the round bottom flask with 5-mL methylene chloride and add to collection tube.
11. Place the 15-mL collection tube on N-Evaporator with water temperature set at 40 °C. Evaporate the sample to near dryness.
12. Remove tube from N-Evaporator and carefully add 1.0 mL of methylene chloride and 10 µL of 0.5 pg/µL (ppb) ISTD solution into the collection tube.

13. Vortex and transfer solution into an autosampler vial.

14. Cap and store vial in -5°C freezer until analysis.

Analytes are identified by their retention times (RTs) and selected ions, and quantified on the pre-defined quantification ion (m/z) and adjusted to the ISTD. RTs of pesticides and the characteristic mass fragments were established from the commercially available pesticide mass spectral library (Agilent Technologies Retention Time Locking Pesticide MS Library, G1049).

Method Calibration

Five levels of standards are prepared in matrix and reagent grade water to calibrate the analysis method. Take reagent water and add 10–15 grams of NaCl in a separatory funnel. Follow the sample preparation steps then concentrate it to 1.0 mL. Take 0.5 mL of the extract from previous step and add 0.5 mL of the standards in acetone. To this, 10 μL of the ISTD solution was added before analysis on the GC/MSD. A linear regression is used including the origin (0,0). The R^2 value should be ≥ 0.99 . Standards are run with the sample set to check for calibration integrity. Continuing calibration standard values should be within $\pm 25\%$ of calibration. Residue concentration is taken from instrument report and calculated.

$$R \text{ (ppt)} = I \times \frac{1000 \text{ g}}{W \text{ (g)}}$$

R = Residue amount (ppt)

I = Instrument response from the report

W = Weight of sample (g)

If the R^2 value of the calibration curve is < 0.99 , the pesticide level may be determined by direct comparison of the residue response to the average response of the nearest bracketing standard concentration. Response of bracketing standards should not vary more than 25%. The residue response should fall within $\pm 30\%$ of standard response. If the residue amount falls outside the calibration curve, the sample will be diluted and reanalyzed. A nonlinear calibration may be necessary to achieve low detection limits (DLs) or address specific instrumental techniques. Nonlinear calibration is not to be used to compensate for detector saturation or to avoid instrument maintenance.

Calibration using single point comparisons:

$$S \text{ (ppt)} = SR/AR \times \frac{1000 \text{ g}}{W \text{ (g)}} \times SA \text{ (ppt)}$$

S = Sample amount

SR = Sample response

AR = Average response of bracketing standards

W = Weight of samples (g)

SA = Standard amount (ppt)

Surrogate and concentration: chlorpyrifos methyl, 500 ppt

Results and Discussion

The LOQ was specified by the TMDL program. The minimum detection level (MDL) for the method is verified by demonstrating the identifiable instrument (GC/MSD) responses. One-liter tap water is fortified with different volumes of the spiking cocktail solution. Three samples were prepared each day representing three different fortification levels (1, 3, and 5) and immediately extracted and assayed. Three sets of experiment were conducted on 3 different days to reflect time variation. The recovery results for each level over 3 days are shown in Table 1 and Figure 1. The recovery results for each day at all three levels are shown in Table 2 and Figure 2. The results for Tables 1 and 2 are the same, but presented in two different ways. Typical recoveries for the compounds are 80%–90%. Eptam (EPTC) shows the worst recovery in the 40%–60% range.

Table 1. The Recovery Results of 17 Target Compounds and the ISTD at Three Levels Over 3 Days

Compound	Level 1 at LOQ			Level 3 at 4x LOQ			Level 5 at 20x LOQ		
	Conc. (ppt)	3-day avg. Recovery	% RSD	Conc. (ppt)	3-day avg. Recovery	% RSD	Conc. (ppt)	3-day avg. Recovery	% RSD
EPTC (Eptam)	50	70	34	200	48	27	1000	62	23
Simazine	200	88	10	800	81	16	1200	86	13
Diazinon	20	92	15	100	80	19	1000	83	13
Dislifton	20	82	13	100	71	21	1000	78	18
Chlorpyrifos methyl	50	87	12	200	78	18	1000	81	15
Carbaryl	20	89	15	100	80	12	1000	88	9
Metolachlor	20	89	9	100	85	14	1000	86	13
Chlorpyrifos	10	94	12	50	81	18	500	85	14
Cyanazine	50	91	11	200	85	17	1000	87	13
Dacthal (DCPA)	50	89	11	200	83	14	1000	85	12
Methodathion	30	90	18	100	85	13	1000	86	18
Propargite	500	84	24	1000	85	12	2000	85	18
Bifenthrin	50	85	15	200	91	11	1000	86	15
Azinphos methyl	50	91	26	200	113	31	1000	89	30
I-Cyhalothrin	100	85	14	400	90	11	1000	86	24
Cyfluthrins	200	89	23	800	91	16	1200	89	24
Cypermethrins	200	87	20	800	90	11	1200	90	30
Esfenvalerate	50	87	30	200	94	15	1000	87	30

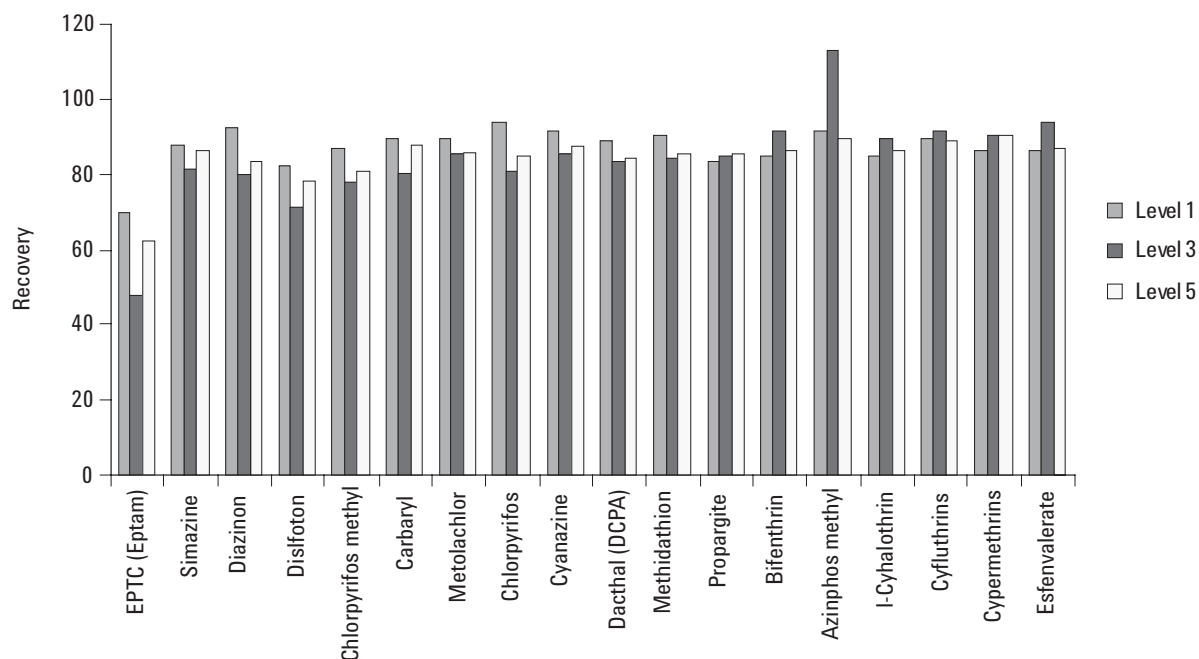


Figure 1. The recoveries of 17 target compounds and the ISTD at three concentration levels over 3 days. (Data from Table 1.)

Table 2. The Recovery Results of 17 Target Compounds and the ISTD on Each Day From All Three Levels

Single day variation (3 levels)	Day 1			Day 2			Day 3		
Compound	Average	SD Dev.	% RSD	Average	SD Dev.	% RSD	Average	SD Dev.	% RSD
EPTC (Eptam)	44	1	3	73	12	16	62	22	36
Simazine	80	1	1	98	2	2	78	8	10
Diazinon	75	2	2	99	5	5	81	13	16
Dislfoton	69	2	3	92	3	4	71	12	16
Chlorpyrifos methyl	75	3	4	95	3	3	76	9	12
Carbaryl	83	6	7	97	7	7	78	4	5
Metolachlor	81	2	2	99	0	0	81	6	7
Chlorpyrifos	81	5	6	101	6	6	78	10	13
Cyanazine	81	3	4	102	2	1	81	6	8
Dacthal (DCPA)	78	2	3	97	1	1	82	6	7
Methidathion	82	3	4	103	6	5	76	1	2
Propargite	80	6	7	101	5	5	73	7	10
Bifenthrin	82	7	8	101	2	2	80	4	4
Azinphos methyl	79	5	6	115	9	8	100	44	44*
I-Cyhalothrin	79	7	8	103	6	6	79	3	4
Cyfluthrins (Total)	84	10	11	109	4	4	75	6	7
Cypermethrins (Total)	80	7	9	110	11	10	77	6	7
Esfenvalerate	88	16	18	109	8	7	70	12	17

*There was some contamination which interfered with the quantitation on the level 5 analysis.

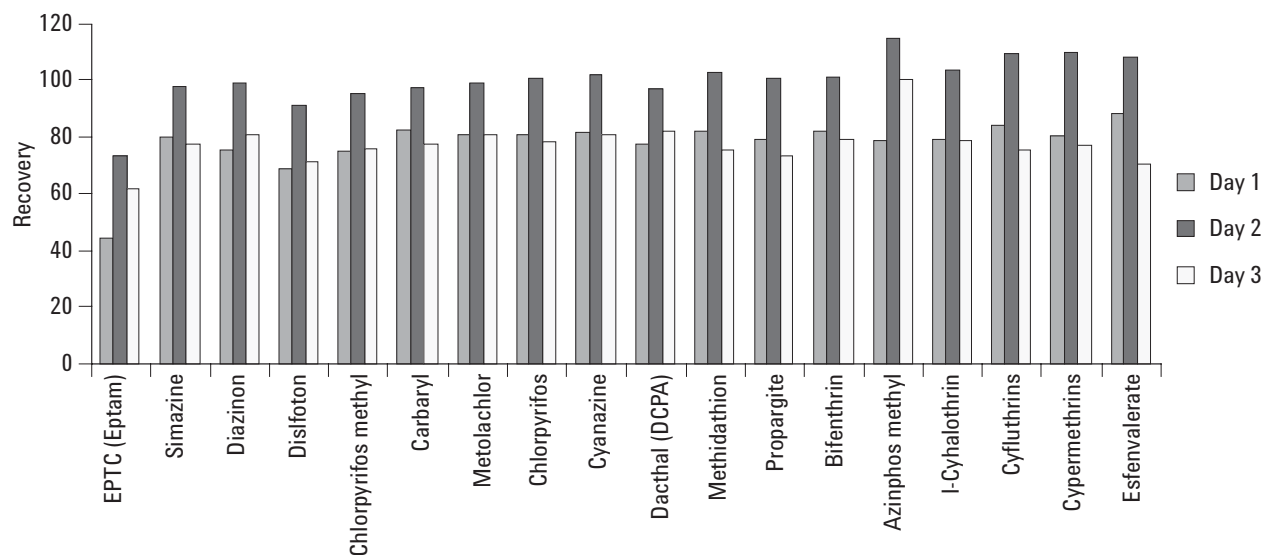


Figure 2. The recovery results of 17 target compounds and the ISTD on each day from all three levels. (Data from Table 2.)

For three chemicals (diazinon, cyanazine, and esfenvalerate), different quantitative ions are used than what was reported in literature to address the matrix effect and the required sensitivity. For MDL demonstration, we were unable to clearly identify azinphos-methyl due to matrix interference. This matrix interference is random and would not always affect the results. Figure 3 shows an overlay of total ion chromatograms (TICs) for the level 5 standards and a water sample in SIM mode.

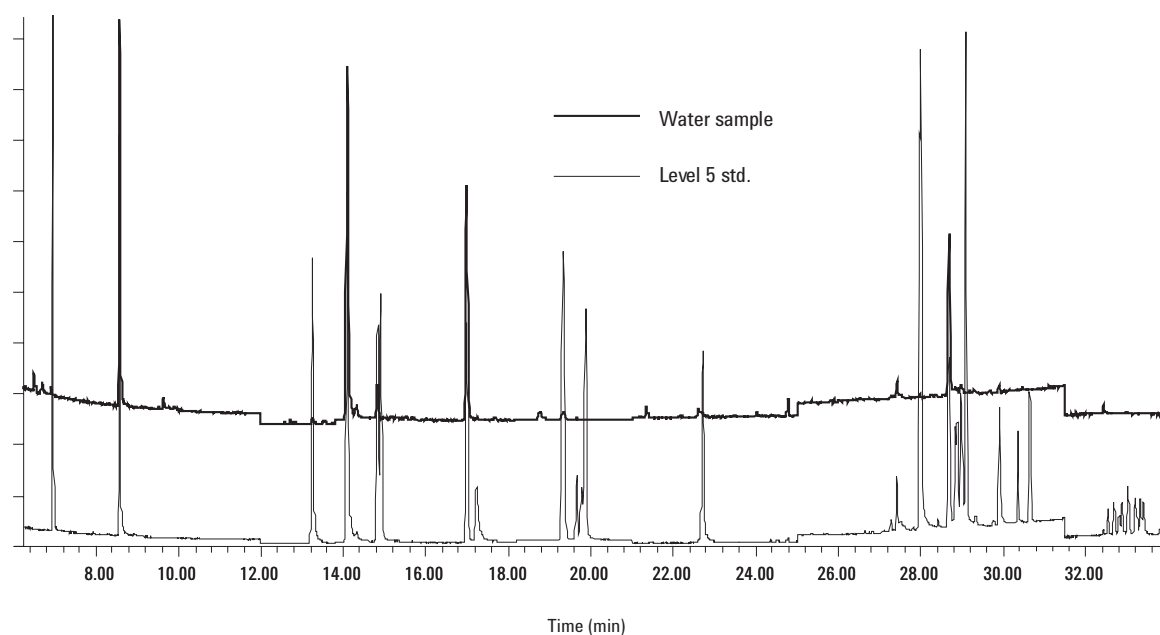


Figure 3. TICs of the level 5 standards and a water sample in SIM mode.

Table 3 summarizes the limit of detection (LOD) and LOQ of the pesticides monitored. The lowest LOQ is 10 ppt for chlorpyrifos.

Table 3. LOD and LOQ of the Pesticides Monitored

Compound	LOD (ppb)	LOQ (ppb)
Eptam (EPTC)	0.020	0.05
Simazine	0.005	0.20
Diazinon	0.007	0.02
Disulfoton	0.007	0.02
Carbaryl	0.007	0.02
Metalochlor	0.007	0.02
Chlorpyrifos	0.004	0.01
Cyanazine	0.007	0.05
Dacthal (DCPA)	0.007	0.05
Methidathion	0.010	0.03
Propargite	0.150	0.50
Bifenthrin	0.007	0.05
Azinphos methyl	0.007	0.05
I-Cyhalothrin	0.030	0.10
Cyfluthrins (4)	0.070	0.20
Cypermethrins (4)	0.070	0.20
Esfenvalerate	0.007	0.05

Surrogate: Chlorpyrifos methyl at 0.5 ng/mL (500 ppt)

ISTD: Anthracene-d₁₀, Pyrene-d₁₀, and Chrysene-d₁₂ at 0.5 ng/mL (500 ppt)

Conclusion

A USGS analytical method using GC/MSD was modified and used to identify the source of pesticides and to calculate the pesticide load in river water during storm seasons. The method was validated to cover 12 organophosphate pesticides and 5 pyrethroids. The LOQs for these target compounds are 10–500 ppt using a GC/MSD SIM method.

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